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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/791,074	03/02/2004	John J. Dunn	BSA 02-16	7913

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BROOKHAVEN SCIENCE ASSOCIATES/
BROOKHAVEN NATIONAL LABORATORY
BLDG. 475D - P.O. BOX 5000
UPTON, NY 11973

EXAMINER	
SHIBUYA, MARK LANCE	
ART UNIT	**PAPER NUMBER**
1639	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	01/30/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/791,074	DUNN ET AL.	
	Examiner	Art Unit	
	Mark L. Shibuya, Ph.D.	1639	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *10/2/06*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-94* is/are pending in the application.

4a) Of the above claim(s) *19,21-57 and 64-94* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-18,20 and 58-63* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *See Continuation Sheet*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Continuation of Attachment(s) 3). Information Disclosure Statement(s) (PTO/SB/08), Paper No(s)/Mail Date :7/6/04; 8/11/04; 4/10/06; and 9/18/06.

DETAILED ACTION

1. Claims 1-94 are pending. Claims 19, 21-57 and 64-94 are withdrawn. Claims 1-18, 20 and 58-63 are examined.

Election/Restrictions

2. Applicant's election with traverse of Group I, claims 1-22 and 58-63 in the reply filed on 10/2/2006 is acknowledged. The traversal is on the ground(s) that claims 50-55 comprise a separate invention, and are distinct from the invention of Group I. This is found persuasive and therefore the inclusion of claims 50-55 in Group I are withdrawn. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement for the restriction of the inventions of the other groups, the election of group is considered to be an election without traverse (MPEP § 818.03(a)).

Applicant's election with traverse of single binding pair, NlaIII, MmeI, biotin/avidin , magnetic beads, identical to, pyrosequencing, soil sample, upstream or downstream of the gene of focus, rDNA genes of eubacteria, one fragmenting enzyme, SmaI and XmaI16 fold degenerate sequence, in the reply filed on 10/2/2006 is acknowledged. The traversal is on the ground(s) that an election of species should not be required between claimed species that are considered clearly unpatentable over each other; and because the elements are interchangeable. This is found persuasive for all species, except for the species of "soil sample". This is not found persuasive for the species of soil sample, because the different samples have different complexity and organismic

diversity, with is a material difference, (see, also below rejection under 35 USC 112, first paragraph), that would require search in different prior art sources and would constitute an undue administrative burden. Therefore, the requirement for election of species is withdrawn for all species, except for the species of soil sample.

The requirement is still deemed proper and is therefore made FINAL.

3. Claims 23-57 and 64-94 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected Invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 10/2/2006.

4. Claims 19, 21 and 22 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected species, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 10/2/2006.

Priority

5. This application, filed 3/2/2004, states that it is a continuation-in-part of 10/113,916, filed 4/1/2002, expressly abandoned 2/7/2006.

6. The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional

application or provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of the first paragraph of 35 U.S.C. 112. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994).

The disclosure of the prior-filed application, Application No. 10/113,916, fails to provide adequate support or enablement in the manner provided by the first paragraph of 35 U.S.C. 112 for one or more claims of this application. Application No. 10/113,916 does not appear to provide support for an invention that is a method for analyzing the organismic complexity of a sample, and comprising relating the listing of signature tags to DNA sequences in database to determine the variety and relative numbers of organisms originally present in the sample, as in claim 1. Therefore, the invention of the claims examined herein, is afforded priority to the filing date of instant application, i.e., 3/2/2004.

Information Disclosure Statement

7. The information disclosure statements (IDS), submitted on 7/6/2004; 8/11/2004; 4/10/2006; and 9/18/2006 have been considered by the examiner.

Specification

8. The reference to Figure 3 in the first line of para [0015] on p. 6 of the specification should be to –Figure 3A and 3B–.

Claim Rejections - 35 USC § 112

9. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

10. Claims 1-18, 20 and 58-63 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This rejection is for lack of written description.

Vas-Cath Inc. v. Mahurkar, 19USPQ2d 1111, clearly states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed." (See page 1117.) The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed." (See Vas-Cath at page 1116).

One of skill in the art cannot envision that the applicant possessed a method for analyzing the organismic complexity of any sample, including complex, environmental communities. The claims recite a method for analyzing the organismic complexity of any sample, using a method that the instant applicant describes as based on "Genome signature Tags", (GST).

The publication of van der Lelie et al., Applied and Environmental Microbiology, Mar. 2006, Vol. 72, No. 3, pp. 2092-2101, (IDS, entered 4/10,2006), teaches that most environmental communities are far too complex to be fully sequenced using metagenome shotgun sequencing. For example, van der Lelie et al. teach that most marine communities have on the order of 100-200 species per ml of water; and that soil communities are even more complex, with an estimated species richness on the order of 4,000 species per gram of soil. The publication of van der Lelie report that the method of genome signature tags (GSTs), (which describes or reads on the instant claimed invention) in in silico, i.e., computer simulated, analysis of 168 entries in the current NCBI database of completely sequenced genomes indicated that "in many cases the individual GST sequences provided sufficient specificity for species identification." van der Lelie et al., state that because they were unable to identify a universal fragmenting enzyme that would generate a limited number of tags from all listed genome, they "decided to devise a modified approach that uses conserved gene sequences in place of the requirement for a fragmenting enzyme." Using in silico analysis of a *defined* microbial consortium, van der Lelie et al., state

> . . . As has been documented for other PCR-based methods, amplification biases lead to a misrepresentation of the overall community composition. It was concluded that the great strength in this technology lies in its discriminatory power. Given its open architecture, diverse application, and the facility with which we can link tags to any gene of interest, the use of SP-GSTs has great potential and application for identifying and analyzing closely related species or strains and simple microbial communities.

van der Lelie et al., at p. 2100.

Thus van der Lelie et al., teach that PCR-methods, such as that of the instant claimed invention, misrepresents the diversity of samples of community composition. van der Lelie et al., teach that the environmental communities are sufficiently diverse that one of skill in the art would not be able to predictably measure the diversity of samples thereof. Therefore, the examiner respectfully submits that applicant's claimed scope of analyzing the organismic complexity of any diverse sample represents only an invitation to experiment.

The specification, at p. 27-29, presents computer-simulated, i.e., in silico, analysis of two organisms. The specification, at p. 27, states to obtain a reasonable estimate of the organismic complexity of a sample, one must develop various strategies for assuring that a statistically significant number of tags have been sequenced. The specification contemplates various possible strategies for addressing these limitation, but does not provide specific working embodiments, thereof. The specification at p. 31, states that the use of a rare-cutting fragmenting enzyme will not always serve the purpose of reducing the number of GSTs generated from organisms and would certainly increase the likelihood of failing to detect some of the organisms comprising a sample.

See Fiers v. Revel, 25 USPQ2d 1601 at 1606 (CAFC 1993) and Amgen Inc. v. Chugai Pharmaceutical Co. Ltd., 18 USPQ2d 1016.

One cannot describe what one has not conceived. See Fiddes v. Baird, 30 USPQ2d 1481 at 1483. In Fiddes, claims directed to mammalian FGF's were found to be unpatentable due to lack of written description for that broad class. The specification provided only the bovine sequence.

Therefore, only methods of analyzing defined samples, but not the full breadth of the claim meets the written description provision of 35 U.S.C. §112, first paragraph. Applicant is reminded that Vas-Cath makes clear that the written description provision of 35 U.S.C. §112 is severable from its enablement provision (see page 1115).

11. Claims 1-18, 20 and 58-63 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for identifying and analyzing closely related species or strains and simple, defined microbial communities, does not reasonably provide enablement for analyzing the organismic complexity of any sample, including diverse, complex environmental samples. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

There are many factors be considered when determining whether there is sufficient evidence to support a determination that a disclosure does not satisfy the enablement requirement and whether undue experiment is necessitated. These factors can include, but are not limited to:

(1) the breadth of the claims;
(2) the nature of the invention;
(3) the state of the prior art;
(4) the relative skill of those in the art;
(5) the level of predictability in the art;
(6) the amount of direction provided by the inventor;
(7) the existence of working examples; and
(8) the quantity of experimentation needed to make or use the invention based on the content of the disclosure.

In re Wands, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

(1 and 2) The breadth of the claims and the nature of the invention: The claims recite a method for analyzing the organismic complexity of any sample, using a method that the instant applicant describes as based on "Genome signature Tags", (GST).

(3 and 5) The state of the prior art and the level of predictability in the art: The publication of van der Lelie et al., Applied and Environmental Microbiology, Mar. 2006, Vol. 72, No. 3, pp. 2092-2101, (IDS, entered 4/10,2006), teaches that most environmental communities are far too complex to be fully sequenced using metagenome shotgun sequencing. For example, van der Lelie et al. teach that most marine communities have on the order of 100-200 species per ml of water; and that soil communities are even more complex, with an estimated species richness on the order of 4,000 species per gram of soil. The publication of van der Lelie report that the method of genome signature tags (GSTs), (which describes or reads on the instant claimed invention) in in silico, i.e., computer simulated, analysis of 168 entries in the current NCBI database of completely sequenced genomes indicated that "in many cases the individual GST sequences provided sufficient specificity for species identification." van der Lelie et al., state that because they were unable to identify a universal fragmenting enzyme that would generate a limited number of tags from all listed genome, they "decided to devise a modified approach that uses conserved gene sequences in place of the requirement for a fragmenting enzyme." Using in silico analysis of a *defined* microbial consortium, van der Lelie et al., state

> . . . As has been documented for other PCR-based methods, amplification biases lead to a misrepresentation of the overall community composition. It was concluded that the great strength in this technology lies in its discriminatory power. Given its open architecture, diverse application, and

> the facility with which we can link tags to any gene of interest, the use of SP-GSTs has great potential and application for identifying and analyzing closely related species or strains and simple microbial communities.

van der Lelie et al., at p. 2100. Thus van der Lelie et al., teach that PCR-methods, such as that of the instant claimed invention, misrepresents the diversity of samples of community composition. van der Lelie et al., teach that the environmental communities are sufficiently diverse that one of skill in the art would not be able to predictably measure the diversity of samples thereof. Therefore, the examiner respectfully submits that applicant's claimed scope of analyzing the organismic complexity of any sample represents only an invitation to experiment.

(4) The level of one or ordinary skill: The level of skill would be high, most likely at the Ph.D. level. However, such persons of ordinary skill in this art, *given its unpredictability*, would have to engage in undue (non-routine) experimentation to carry out the invention as claimed.

(6-7) The amount of direction provided by the inventor and the existence of working examples: The specification, at p. 27-29, presents computer-simulated, i.e., in silico, analysis of two organisms. The specification, at p. 27, states to obtain a reasonable estimate of the organismic complexity of a sample, one must develop various strategies for assuring that a statistically significant number of tags have been sequenced. The specification contemplates various possible strategies for addressing these limitation, but does not provide specific working embodiments, thereof. The specification at p. 31, states that the use of a rare-cutting fragmenting enzyme will not always serve the purpose of reducing the number of GSTs generated from organisms

and would certainly increase the likelihood of failing to detect some of the organisms comprising a sample.

(8) The quantity of experimentation needed to make or use the invention based on the content of the disclosure: The claims contain only broad recitations of analyzing the organismic complexity of any sample. However, the instant specification does not provide to one skilled in the art a reasonable amount of guidance with respect to the direction in which the experimentation should proceed in carrying out the full scope of the claimed methods. Note that there must be sufficient disclosure, either through illustrative examples or terminology, to teach those of ordinary skill how to make and use the invention as broadly as it is claimed. *In re Vaeck*, 947 F.2d 488, 496 and n.23, 20 USPQ2d 1438, 1455 and n.23 (Fed. Cir. 1991). Therefore, it is deemed that further research of an unpredictable nature would be necessary to make or use the invention as claimed. Thus, due to the inadequacies of the instant disclosure, undue experimentation would be required of one of skill in the art to practice the full scope of the claimed invention.

Claim Rejections - 35 USC § 103

12. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

13. Claims 1-18, 20 and 58-63 are rejected under 35 U.S.C. 103(a) as being unpatentable over **Zhou et al.**, Journal of Applied Microbiology, 2001, Vol. 90, pp. 96-105, and **Dunn et al.**, (11/2002), Genome Research, Vol. 12, pp. 1756-1765, (IDS, entered 7/6/2004).

The claims are drawn to a method for analyzing the organismic complexity of a sample, comprising a method that is a genome signature tags (GST) procedure, which involves the initial digestion of genomic DNA with two type II restriction enzymes. After the digestion with the first enzyme, the cut ends are modified with a first member of a specific binding member, such as biotin, to allow their solid phase affinity capture after treatment with the second enzyme. A linker containing a MmeI recognition site is ligated to the nonbiotinylated ends, and MmeI digestion is then used to liberate 21-bp GST sequences from the un-tethered ends of the captured fragments. The released

monomeric GSTs are PCR-amplified and randomly ligated on themselves prior to cloning and sequencing.

Thus the claims comprise a method for analyzing the organismic complexity of a sample, comprising: **a**) providing a sample containing one or more organisms; **b**) isolating the DNA from the organisms in the sample; **c**) contacting the DNA with a fragmenting enzyme, said fragmenting enzyme being a type II restriction endonuclease, under conditions appropriate for substantially complete digestion of the DNA thereby generating a plurality of DNA fragment species, each having complementary cohesive termini; **d**) incubating the DNA fragment species of step c) with a molar excess of a capture adapter, the capture adapter being a substantially duplex DNA having a portion which is covalently modified with a first member of a specific binding pair and also having one cohesive end compatible with the cohesive termini generated by the fragmenting enzyme of step c), under conditions appropriate for ligating the capture adapter to each of the complementary cohesive termini of the DNA fragment species, thereby generating a plurality of ligation products; **e**) contacting the ligation products of step d) with an anchoring enzyme under conditions for substantially complete digestion of the ligation products, said anchoring enzyme being a restriction endonuclease having a high probability of cleaving a substantial number of DNA fragment species generated in step c) at least one time, thereby generating a plurality of digestion products which have one cohesive terminus generated by the anchoring enzyme and a portion that is covalently modified with a first member of the specific binding pair; **f**) capturing the digestion products of step e) by contacting the digestion products with a solid support

having an attached second member of the specific binding pair; **g**) incubating the solid support and captured digestion products of step f) with a molar excess of a duplex linker having a type IIS restriction enzyme recognition sequence and one cohesive terminus compatible with termini generated by the anchoring enzyme of step e), under conditions appropriate for ligating one duplex linker to the cohesive termini of the captured digestion products, thereby ligating a recognition sequence for a type IIS restriction enzyme to the captured digestion products; **h**) incubating the ligation product of step g) with the type IIS restriction enzyme, under conditions appropriate for substantially complete digestion thereby releasing the duplex linkers, each having an appended signature tag; **i**) recovering the released duplex linkers and appended signature tags; **j**) incubating the recovered linkers and tags of step i) with a molar excess of an amplification adapter, the amplification adapter having one terminus compatible with the termini of the appended signature tags, the incubation being carried out under conditions appropriate for ligating one amplification adapter to each appended signature tag; **k**) recovering the ligation products of step j); **l**) determining the nucleotide sequence of a statistically significant number of appended signature tags to generate a listing of signature tags; and, **m**) relating the listing of signature tags of step i) to DNA sequences in databases to determine the variety and relative numbers of organisms originally present in the sample thereby analyzing the organismic complexity of the sample; and variations thereof.

Zhou et al., Journal of Applied Microbiology, 2001, throughout the publication and at the abstract, and at pp. 99-100, describe a method for analyzing the organismic

complexity of a sample comprising relating the restriction fragment length polymorphism, sequence data, operational taxonomic unit analysis and DNA sequence databases, in order to determine the variety and relative numbers of organisms originally present in the sample thereby analyzing the organismic complexity of the sample.

Zhou et al., do not teach a method that is a genome signature tags (GST) procedure, which involves the initial digestion of genomic DNA with two type II restriction enzymes. After the digestion with the first enzyme, the cut ends are modified with a first member of a specific binding member, such as biotin, to allow their solid phase affinity capture after treatment with the second enzyme. A linker containing a MmeI recognition site is ligated to the nonbiotinylated ends, and MmeI digestion is then used to liberate 21-bp GST sequences from the un-tethered ends of the captured fragments. The released monomeric GSTs are PCR-amplified and randomly ligated on themselves prior to cloning and sequencing.

Dunn et al., (11/2002), Genome Research, Vol. 12, pp. 1756-1765, (IDS, entered 7/6/2004), throughout the publication and at p. 1757, teach a method for analyzing the organismic complexity of a sample, comprising a method that is a genome signature tags (GST) procedure, which involves the initial digestion of genomic DNA with two type II restriction enzymes. After the digestion with the first enzyme, the cut ends are modified with a first member of a specific binding member, such as biotin, to allow their solid phase affinity capture after treatment with the second enzyme. A linker containing a MmeI recognition site is ligated to the nonbiotinylated ends, and MmeI

digestion is then used to liberate 21-bp GST sequences from the un-tethered ends of the captured fragments. The released monomeric GSTs are PCR-amplified and randomly ligated on themselves prior to cloning and sequencing.

It would have been *prima facie* obvious, at the time the invention was made, for one of ordinary skill in the art to have made and used a method for analyzing the organismic complexity of a sample, comprising a method that is a genome signature tags (GST) procedure, which involves the initial digestion of genomic DNA with two type II restriction enzymes. After the digestion with the first enzyme, the cut ends are modified with a first member of a specific binding member, such as biotin, to allow their solid phase affinity capture after treatment with the second enzyme. A linker containing a MmeI recognition site is ligated to the nonbiotinylated ends, and MmeI digestion is then used to liberate 21-bp GST sequences from the un-tethered ends of the captured fragments. The released monomeric GSTs are PCR-amplified and randomly ligated on themselves prior to cloning and sequencing.

One of ordinary skill in the art would have been motivated to combine the methods for analyzing diversity and using genomic signature tags, because Dunn et al., (11/2002), at pp. 1762-63, bridging paragraph, teach that the GST analysis is a direct DNA sequence approach for profiling DNA, that would reduce the need for enrichment and isolation of microbial cultures and that reduces the number of genomic fragments or tags to sequence; and Zhou et al., at pp. 96, bridging paragraph, teach that while the high microbial diversity of environmental samples offer potential use in biotechnology,

the loss of diversity upon enrichment and isolation of microbial cultures, influence and lower the diversity of the cultures, thus pointing to a problem addressed by Dunn.

One of ordinary skill in the art would have had a reasonable expectation of success in combining the methods taught by Zhou and Dunn, because Zhou teaches characterizing diversity of Fe(III)-reducing, thermophilic, enrichment cultures from the deep subsurface, which are dominated by a few low G + C Gram-positive bacteria.

14. Claims 1-18, 20 and 58-63 are rejected under 35 U.S.C. 103(a) as being unpatentable over **Zhou et al.**, Journal of Applied Microbiology, 2001, Vol. 90, pp. 96-105, and **Dunn et al.**, US 2003/0186251 A1, (published 10/2/2003).

Zhou et al., is relied upon, as above.

Zhou et al., do not teach a method that is a genome signature tags (GST) procedure, which involves the initial digestion of genomic DNA with two type II restriction enzymes. After the digestion with the first enzyme, the cut ends are modified with a first member of a specific binding member, such as biotin, to allow their solid phase affinity capture after treatment with the second enzyme. A linker containing a MmeI recognition site is ligated to the nonbiotinylated ends, and MmeI digestion is then used to liberate 21-bp GST sequences from the un-tethered ends of the captured fragments. The released monomeric GSTs are PCR-amplified and randomly ligated on themselves prior to cloning and sequencing.

Dunn et al., US 2003/0186251 A1, (published 10/2/2003), throughout the publication and especially at para [0008]-[0009], and Fig. 1, teach a method for analyzing the organismic complexity of a sample, comprising a method that is a genome signature tags (GST) procedure, which involves the initial digestion of genomic DNA with two type II restriction enzymes. After the digestion with the first enzyme, the cut ends are modified with a first member of a specific binding member, such as biotin, to allow their solid phase affinity capture after treatment with the second enzyme. A linker containing a MmeI recognition site is ligated to the nonbiotinylated ends, and MmeI digestion is then used to liberate 21-bp GST sequences from the un-tethered ends of the captured fragments. The released monomeric GSTs are PCR-amplified and randomly ligated on themselves prior to cloning and sequencing.

It would have been *prima facie* obvious, at the time the invention was made, for one of ordinary skill in the art to have made and used a method for analyzing the organismic complexity of a sample, comprising a method that is a genome signature tags (GST) procedure, which involves the initial digestion of genomic DNA with two type II restriction enzymes. After the digestion with the first enzyme, the cut ends are modified with a first member of a specific binding member, such as biotin, to allow their solid phase affinity capture after treatment with the second enzyme. A linker containing a MmeI recognition site is ligated to the nonbiotinylated ends, and MmeI digestion is then used to liberate 21-bp GST sequences from the un-tethered ends of the captured fragments. The released monomeric GSTs are PCR-amplified and randomly ligated on themselves prior to cloning and sequencing.

One of ordinary skill in the art would have been motivated to combine the methods for analyzing diversity and using genomic signature tags, because Dunn et al., US 2003/0186251 A1, at para [0017], teach that the GST analysis is a direct DNA sequence approach for profiling DNA, that would reduce the need for enrichment and isolation of microbial cultures and that reduces the number of genomic fragments or tags to sequence; and Zhou et al., at pp. 96, bridging paragraph, teach that while the high microbial diversity of environmental samples offer potential use in biotechnology, the loss of diversity upon enrichment and isolation of microbial cultures, influence and lower the diversity of the cultures, thus pointing to a problem addressed by Dunn.

One of ordinary skill in the art would have had a reasonable expectation of success in combining the methods taught by Zhou and Dunn, because Zhou teaches characterizing diversity of Fe(III)-reducing, thermophilic, enrichment cultures from the deep subsurface, which are dominated by a few low G + C Gram-positive bacteria.

15. Claims 1-18, 20 and 58-63 are rejected under 35 U.S.C. 103(a) as being unpatentable over **Zhou et al.**, Journal of Applied Microbiology, 2001, Vol. 90, pp. 96-105, **Li et al.**, (US 2003/0165923 A1, filing date 1/20/2000) (priority to Provisional application No. 60/215,596, 1/20/2000), and **Velculescu et al.** (US 6,498,013 B1, filing date 7/28/2000) (priority to Provisional application No.s 60/221,556, 7/28/2000 and 60/233,431, 9/18/2000)

Zhou et al., is relied upon, as above.

Zhou et al., do not teach a method that is a genome signature tags (GST) procedure, which involves the initial digestion of genomic DNA with two type II restriction enzymes. After the digestion with the first enzyme, the cut ends are modified with a first member of a specific binding member, such as biotin, to allow their solid phase affinity capture after treatment with the second enzyme. A linker containing a MmeI recognition site is ligated to the nonbiotinylated ends, and MmeI digestion is then used to liberate 21-bp GST sequences from the un-tethered ends of the captured fragments. The released monomeric GSTs are PCR-amplified and randomly ligated on themselves prior to cloning and sequencing.

Li et al., (US 2003/0165923 A1) (priority to Provisional application No. 60/215,596, 1/20/2000), throughout the publication and particularly at para [004] teach establishing differences between DNA samples from two different sources, and refer to DNA fingerprinting for purposes of comparison. Li et al., at para [0016]-[0018], teach:

> [0016] In one aspect, the present invention provides a method for the simultaneous sequence-specific identification and separation of polynucleotide fragments in a polynucleotide population comprising the steps of, preferably converting RNA to DNA and: (a) digesting the polynucleotide population with one or more restriction endonucleases . . . (b) ligating the restriction fragments having the same overhangs to a series of adapters whose sequences are complementary to the overhangs; and (c) amplifying the restriction fragments. The polynucleotide may be genomic DNA . . .
>
> [0017] In another aspect, the invention provides a method further comprising the step of digesting the restriction fragments obtained in step (a) with one or more further restriction endonucleases producing restriction fragments with single-stranded overhangs different from those produced in step (a). The single-stranded overhangs produced are ligated to adapters whose sequences are complementary to the overhangs.

> [0018] In yet another aspect, the restriction fragments produced in the method are amplified by polymerase chain reaction (PCR). The PCR primers are designed from the adapters . . . The PCR products may be isolated, sequenced and cloned into a vector. The vector may be transformed into a host cell in order to express the cDNA and produce a polypeptide. Accordingly, the present invention also provides a host cell transformed with the vector.

US 2003/0165923 A1 at p. 3. Thus Li et al. teach digesting genomic DNA with one or more restriction endonucleases, ligating restriction fragments with the same overhands to a series of adapters, amplifying the restriction fragments, digesting the restriction fragments with different restriction endonucleases and cloning the amplification products into vectors to form libraries.

Li et al., state:

> [0069] Yet another aspect provides a method for detecting genetic markers comprising obtaining genomic DNA, which includes plastid or mitodchondrial DNA, from a first source having a first phenotype or trait of interest. The DNA can be obtained from any organism, for example, a bacteria, yeast, fungus, plant or animal. In one embodiment, the organism is further characterized as being from a particular strain, breed, line or cultivar. Likewise, any phenotype or trait of interest can be used, for example, growth rate, virulence, abiotic or biotic stress resistance or susceptability, grain quality, feed conversion, disease resistance or susceptability, etc. The DNA is digested with at least one restriction endonuclease having a degenerate recognition or cleavage sequence comprising at least one degenerate base represented by the formula N^m, where N is the extent of degeneracy, and m is the number of degenerate bases. In one embodiment, N can be an integer between 2 and 4 and m is between 1 and 5. In another embodiment, the endonucleases used are three base cutters, four base cutters, five base cutters, six base cutters, seven base cutters, eight base cutters, or any combination thereof. As will be recognized by those skilled in the art, the digestion with endonuclease digestions can be carried out simultaneously, sequentially, or a combination of both, e.g. digestions with several groups of enzymes conducted sequentially.

Li et al., at para [0069].

Li et al., does not teach incubating the fragments with biotinylated adapters; contacting the product with the restriction enzyme NlaIII; recovering the labelled NlaIII restriction fragments with streptavidin-coated magnetic beads; incubating the magnetic beads bearing recovered labelled fragments with a duplex linker having NlaIII cohesive termini for ligation of complementary cohesive termini, thereby generating recognition sequences for the restriction enzyme MmeI at any location in which the duplex linker is ligated to an NlaIII cohesive termini; incubating the magnetic beads with bound recovered labelled fragments with the restriction enzyme MmeI to release a fragment comprising the duplex linker and an appended genomic signature tag; incubating this fragment with an adapter fragment, the adapter fragment having a 16-fold degenerate overhang, the adapter fragment adding two consecutive T residues and a second NlaIII restriction enzyme recognition sequence following ligation to a free MmeI restriction enzyme-generated end, incubation being carried out under conditions appropriate for ligation of the adapter fragment to any recovered fragment having a free MmeI restriction enzyme-generated end; amplifying the ligation products with a pair of biotinylated primers specific for the duplex linker and the adapter fragment; incubating the amplification product with the restriction enzyme NlaIII; capturing biotinylated end fragments generated using streptavidin-coated magnetic beads leaving tag fragments, comprising 19-base pair duplex genomic signature tags with NlaIII cohesive end tags, free in solution; isolating the tag fragments; and ligating the isolated tag fragments to form concatemers.

Velculescu et al. (US 6,498,013 B1) (priority to Provisional application No.s 60/221,556, 7/28/2000 and 60/233,431, 9/18/2000), throughout the patent and particularly at col. 6, line 31-col. 7, line 3, Figures 1A and 1B, 5A and 5B, teach a SAGE serial analysis of gene expression) method of incubating the fragments with biotinylated adapters and contacting the product with an "anchoring enzyme" that is the restriction enzyme NlaIII, recovering the labelled NlaIII restriction fragments with streptavidin-coated magnetic beads; at col. 8, line 3-col. 9, line 17, teach incubating the magnetic beads bearing recovered labelled fragments with a duplex linker having NlaIII cohesive termini for ligation of complementary cohesive termini, thereby generating recognition sequences for a "tagging enzyme" that is the restriction enzyme MmeI, at any location in which the duplex linker is ligated to an NlaIII cohesive termini; incubating the magnetic beads with bound recovered labelled fragments with the restriction enzyme MmeI to release fragment comprising the duplex linker and an appended genomic signature tag with a defined sequence tag of 6 to 30 base pairs, of 9-15 base pairs or 19-30 base pairs; at col. 5, line 52-col. 6, line 7, col. 9, lines7-18, teach incubating this fragment with linkers that are adapter fragments, the adapter fragment having a 16-fold degenerate overhang in order to duplex with the genomic signature tag, absent evidence to the contrary, the adapter fragment adding base pair 3'-overhanging ends and a second NlaIII restriction enzyme recognition sequence following ligation to a free MmeI restriction enzyme-generated end (see Figures 5A and 5B), incubation being carried out under conditions appropriate for ligation of the adapter fragment to any recovered fragment having a free MmeI restriction enzyme-generated end; amplifying the ligation

products with a pair of biotinylated primers specific for the duplex linker and the adapter fragment; incubating the amplification product with the restriction enzyme NlaIII; at col. 9, line 66-col. 10, line 15, col. 10, line 61-col. 11, line 17 and Figures 5A and 5B, teach leaving tag fragments, comprising 18-20 nucleotide base pair duplex genomic signature tags with NlaIII cohesive end tags, free in solution; isolating the tag fragments; ligating the isolated tag fragments to form concatemers; and, at col. 9, line 66-col. 10, line 56, inserting the concatemer into vectors to produce a library (see Table 1).

It would have been *prima facie* obvious at the time the invention was made for one of ordinary skill in the art to have combined a method for analyzing the organismic complexity of a sample, as taught by Zhou et al., with a method for the genomic tagging, as taught by Li et al., and with the expressed sequence tagging method of Velculescu, wherein said method of Velculescu used the restriction enzyme NlaIII as an anchoring enzyme to cleave fragmented genomic DNA, followed by ligation to adapters to produce MmeI restriction site, followed by cleavage with MmeI restriction enzyme as a tagging enzyme, and for using biotinylated linkers for linker removal at later stages of tag production.

One of ordinary skill in the art would have been motivated to combine the methods for analyzing diversity and using genomic signature tags, because Zhou et al., at pp. 96, bridging paragraph, teach that while the high microbial diversity of environmental samples offer potential use in biotechnology, the loss of diversity upon enrichment and isolation of microbial cultures, influence and lower the diversity of the cultures, and the methods of Li et al., and Velculescu et al., suggest a direct DNA

sequence approach for profiling DNA, that would reduce the need for enrichment and isolation of microbial cultures and that reduces the number of genomic fragments or tags to sequence.

One of ordinary skill in the art would have been motivated to use the expressed sequence tagging method, because Velculescu teaches:

> One of the primary strengths of using a restriction endonuclease as a tagging enzyme which cuts at least 17 or 18 nucleotides distant from its recognition site is the ability to unambiguously identify a location in the genome from which a long tag is derived. Thus, it is significantly easier and more accurate to determine the identity of the gene or genomic region that gave rise to a tag, particularly if one is dealing with an organism for which significant genomic data but only limited cDNA sequence information is available. Table 4 shows a computation of the probability that tags of differing length will be unique in the human genome. In addition, a comparison of the number of times long tags vs their cognate short tags "hit" the human genome is shown in FIG. 5. This analysis is based on theoretical tags derived from known genes on Chromosome 22.
>
> In one embodiment, a sequence tag for a sample is compared to corresponding information in a sequence database to identify known sequences that match the sample sequence. Preferably the database is genomic sequence, more preferably human genomic sequence. One or more tags can be determined for each sequence in the sequence database as the N base pairs adjacent to each anchoring enzyme site within the sequence. However, in the preferred embodiment, only the first anchoring enzyme site from the 3' end is used to determine a tag. In the preferred embodiment, the adjacent base pairs defining a tag are on the 3' side of the anchoring enzyme site, and N is preferably 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29.

Velculescu et al., col. 12, lines 16-40. Also, Velculescu at col. 3, line 60-col. 4, line 45, teaches that improvements involving the use of NlaIII and particularly, MmeI, allow the production of longer tags that permit unambiguous identification of genomic sequences. Also, Velculescu at col. 5, line 54-col. 6, line 7, teaches using a 2 bp 3-overhanging end

to lessen contamination by linker sequences for higher yields; because the instant Specification is silent as to the reasons for inserting two consecutive T residues (i.e., particularly T residues), absent evidence to the contrary, Velculescu's teaching of 2 bp overhangs encompasses two consecutive T residues.

One of ordinary skill in the art would have had a reasonable expectation of success in combining the methods taught by Zhou and Dunn, because Zhou teaches characterizing diversity of Fe(III)-reducing, thermophilic, enrichment cultures from the deep subsurface, which are dominated by a few low G + C Gram-positive bacteria. One of ordinary skill in the art would have had a reasonable expectation of success in using the expressed sequence tag method of Velculescu to analyze fragmented genomic DNA, because said tag method would not distinguish genomic DNA from cDNA.

Conclusion

16. Claims 1-18, 20 and 58-63 are rejected.

17. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Mark L. Shibuya, Ph.D. whose telephone number is (571) 272-0806. The examiner can normally be reached on M-F, 8:30AM-5:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. James Schultz can be reached on (571) 272-0763. The fax phone

number for the organization where this application or proceeding is assigned is 571-273-8300.

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Mark L. Shibuya, Ph.D.
Primary Examiner
Art Unit 1639